Chapter 2

β-CAROTENE (PROVITAMIN A) PRODUCTION WITH ALGAE

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1 HISTORICAL

Dunaliella is a unicellular, biflagellate, naked green alga (Chlorophyceae, Dunaliellales), and the type species of this genus, Dunaliella salina (Dunal) Teodoresco is often found in natural hypersaline waters where it colours the brines red (Teodoresco, 1905). This algal species was first recognised as containing high intracellular concentrations of β-carotene by Mil’ko (1963) and Aasen et al. (1969). Initial research on the potential of using this alga as a commercial source of β-carotene began in the Ukraine in the 1960s (cf. Massyuk, 1966; Massyuk & Abdula, 1969) and it was later also proposed as a commercial source of glycerol (Ben-Amotz, 1980; Chen & Chi, 1981; Ben-Amotz & Avron, 1982).

A number of commercial-scale developments on the production of β-carotene from D. salina have commenced in Australia (Curtain et al., 1987; Borowitzka & Borowitzka, 1988a), Israel (Rich, 1978) and the USA (Klausner, 1986), and a number of open-pond and closed reactor experimental units are in development stages. Pilot projects are also under way in China and Chile. Commercial quantities of extracted algal β-carotene and dried D. salina powder rich in β-carotene have been marketed by companies from the US and Australia since 1985. All are targeting the markets for ‘natural’ food and animal feed colourings, and ‘natural’ β-carotene (provitamin A) nutritional supplements.

2 CHEMICAL AND PHYSICAL PROPERTIES

β-Carotene is accumulated as droplets in the chloroplast stroma of Dunaliella salina cells, particularly when culture conditions include high light intensities,
high temperature, high salinity and deficiency of nitrogen source (Lerche, 1937; Mil’ko, 1963; Mironyuk & Einor, 1968; Semenko & Abdullayev, 1980; Ben-Amotz & Avron; 1983; Borowitzka et al., 1984). β-carotene contents of up to 14% of dry weight have been reported for *D. salina* (Ben-Amotz et al., 1982; Borowitzka et al., 1984). The β-carotene does not appear to act as a light-harvesting photo-accessory pigment, but rather as a photo-protective ‘sunscreen’ to the chlorophyll and cell DNA in the high light intensities which characterise the normal salt-lake environments where this alga grows (MacKinney & Chichester, 1960; Ben-Amotz, 1980). Borowitzka & Borowitzka (1988a) have also proposed that β-carotene acts as a ‘carbon sink’ in *D. salina* for excess combined carbon produced when photosynthesis continues under conditions where growth is limited.

The β-carotene in the chloroplast droplets is a mixture of the cis- and trans-isomers. A typical analysis from *D. salina* (also called *D. bardawil*) gives the following composition as percentages of total β-carotene: 15-cis-β-carotene, 10%; 9-cis-β-carotene, 41%; all-trans-β-carotene, 42%; other isomers, 6% (Ben-Amotz et al., 1982).

For analytical purposes, total β-carotene may be calculated from its extinction in acetone extracts (*E*₅₅₂ at 452 nm is 2592; Bauernfeind, 1981). HPLC is required to separate the isomers, and to characterise the small quantities of other carotenoids also present in typical *D. salina* extracts (Nelis & de Leenheer, 1983). Figure 1 shows an HPLC analysis of a typical commercial batch of 20% β-carotene suspension in peanut oil produced by Western Biotechnology Limited from *D. salina*.

### 3 STRAIN IMPROVEMENT, SELECTION AND MAINTENANCE

*Dunaliella salina* production systems, like those used by Western Biotechnology Ltd (Fig. 2), have large open ponds situated in, or near, salt lakes or solar...